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# **Wnt Signaling Roles on the Structure and Function of the Central Synapses: Involvement in Alzheimer's Disease**

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Nibaldo C. Inestrosa and Lorena Varela-Nallar

Additional information is available at the end of the chapter

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## **1. Introduction**

Wnts compromise a large family of secreted glycoproteins that have shown to be part of the signaling molecules that regulate several aspects of development such as axis formation and midbrain development [1, 2]. In mammals at least 19 Wnt members have been found. The interaction of a Wnt protein with members of the Frizzled (Fz) family of seven-pass transmembrane cell-surface receptors triggers the activation of the Wnt signaling pathway [3-5]. In human and mice, 10 members of the Fz family have been identified. In addition, receptor-like tyrosine kinase (Ryk) and receptor tyrosine kinase-like orphan receptor (Ror2) have been identified as alternative Wnt receptors [6-8]. Different Wnt signaling cascades are activated downstream the Wnt receptors, identified as Wnt/ $\beta$ -catenin or canonical pathway, and  $\beta$ -catenin-independent or non-canonical pathways. The canonical pathway involves the transcription of Wnt target genes, while activation of non-canonical Wnt pathways may induce either an increase in intracellular calcium concentration or activation of the c-Jun-N-terminal kinase (JNK) cascade [3, 9, 10].

The Wnt pathway participates in the development of the central nervous system (CNS) and growing evidence indicate that Wnts also regulates the function of the adult nervous system [11, 12]. In fact, most of the key components including Wnts and Fz receptors are expressed in the adult brain [13, 14]. Wnt ligands have shown to regulate synaptic assembly as well synaptic plasticity and neurotransmission [15-20], and more recently it has also been involved in the adult neurogenesis [21-25].

Deregulation of the Wnt signaling has been associated to several pathologies, been cancer the most widely documented [26-28]. More recently, altered Wnt signaling have been related to mental disorders, mood disorders and neurodegenerative diseases [12, 29-32].

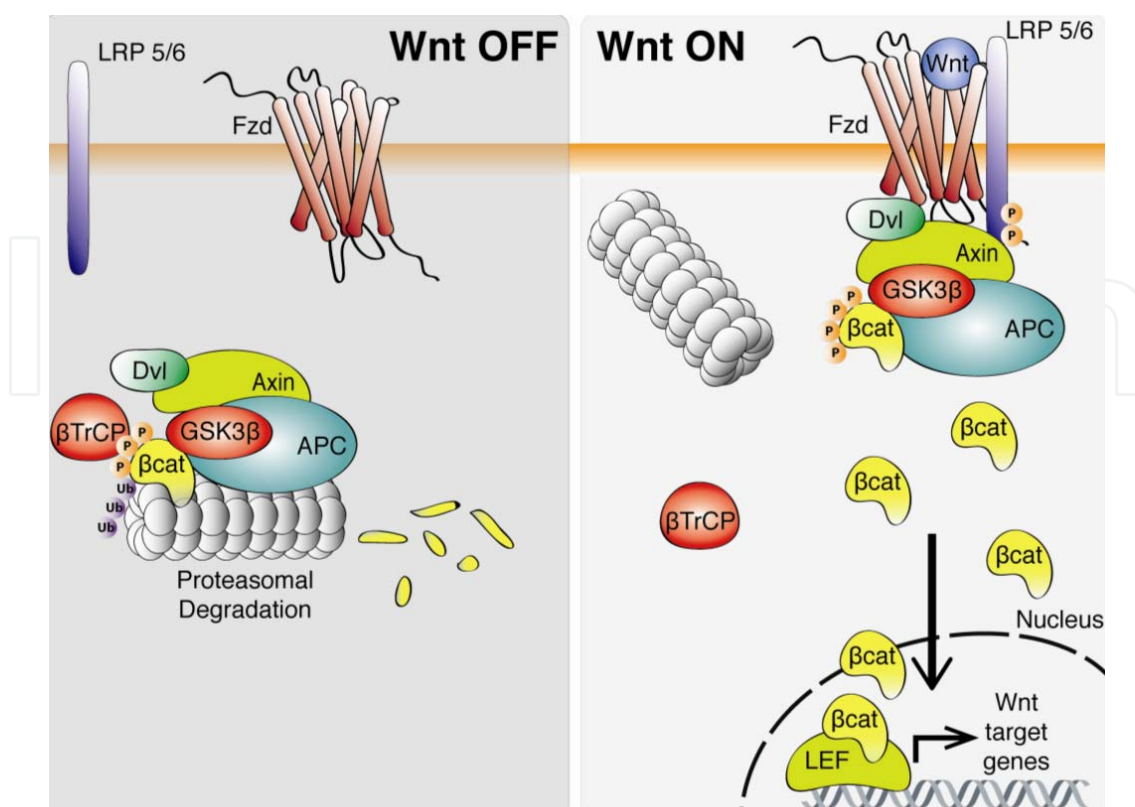
In the first part of this chapter we will address what is currently known about the signaling cascades of canonical and non-canonical pathways. Then, we will review recent findings from our and other labs on the specific effects of different Wnt ligands on the structure of pre- and postsynaptic regions and on glutamatergic neurotransmission in hippocampal neurons. The synaptic role of some Fz receptors will also be reviewed. Finally, the neuroprotective effect of the Wnt signaling activation will be discussed mainly focused on the protection against the toxicity of A $\beta$ -peptide aggregates associated to the pathogenesis of Alzheimer's disease.

## 2. The Wnt signaling pathway: Canonical and non-canonical signaling cascades

The binding of Wnt ligands to Fz receptors can trigger the activation of different signaling cascades. In addition to Fz, other proteins have been described as alternative receptors or co-receptors, such as the low-density lipoprotein receptor-related protein 5 (LRP5), LRP6, Ror1, Ror2 and Ryk [3, 33-36], increasing the complexity of the Wnt signaling activation. It has been suggested that the binding of Wnts to specific receptors/co-receptors may selectively activate distinct signaling pathways.

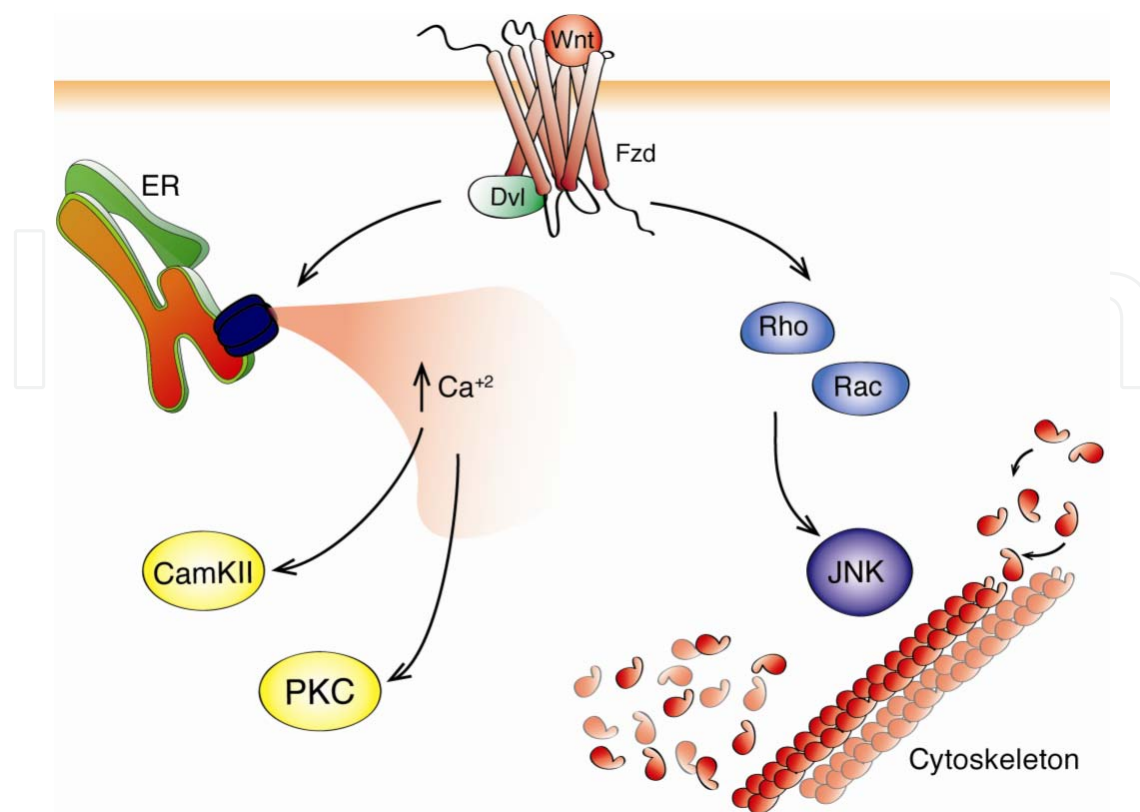
The first Wnt signaling pathway identified was the canonical Wnt/ $\beta$ -catenin pathway (Figure 1). In the absence of Wnt stimulation, the levels of cytoplasmic  $\beta$ -catenin are low since it is ubiquitinated and constantly degraded in the proteasome [37].  $\beta$ -catenin is phosphorylated by casein kinase 1 $\alpha$  (CK1 $\alpha$ ) and glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) in a multiprotein complex composed also of the scaffold protein axin and adenomatous polyposis coli (APC) [38-42]. Phosphorylated  $\beta$ -catenin is recognized by  $\beta$ -TrCP, which is part of an E3 ubiquitin ligase complex, and is ubiquitinated and subsequently degraded [43]. Activation of the Wnt/ $\beta$ -catenin pathway initiated by the binding of a Wnt ligand to a Fz receptor and coreceptors LRP5/6 activates the protein Dishevelled (Dvl) usually by phosphorylation, and triggers the recruitment of axin to the phosphorylated tail of LRP, inhibiting the degradation pathway consequently inducing the cytoplasmic stabilization of  $\beta$ -catenin which enters the nucleus and regulates the transcription of Wnt target genes [28]. Recently, it was shown that when the destruction complex is associated with phosphorylated LRP, it may still capture and phosphorylates  $\beta$ -catenin, but ubiquitination is blocked (Figure 1, right panel) [44].

In the nucleus,  $\beta$ -catenin binds to members of the family of T-cell factor (Tcf) and lymphoid enhancer factor (Lef) [45-47]; this binding displaces Groucho, which is bound to Tcf/Lef and recruits histone deacetylases (HDAC) to repress the transcription of Wnt target genes [48-51]. Several Wnt target genes have been identified including *c-Myc*, *cyclin D1*, *Axin2*, *Calcium/calmodulin-dependent protein kinase type IV* (CamKIV) [52-55]. In addition, by using an *in silico* analysis based on multiple Classification and Regression Tree (CART), 89 new genes were predicted to be targets of the Wnt/ $\beta$ -catenin pathway [56].



**Figure 1. Canonical Wnt/ $\beta$ -catenin signaling pathway.** (Left panel) In the absence of a Wnt protein, GSK-3 $\beta$  phosphorylates  $\beta$ -catenin which targets it for ubiquitination by  $\beta$ -TrCP and degradation in the proteasome. (Right panel) Activation of the signaling pathway by the binding of a Wnt ligand to Fz receptor and coreceptors LRP5/6 triggers the association of the destruction complex with phosphorylated LRP. In this condition, the complex may still capture and phosphorylate  $\beta$ -catenin, however the ubiquitination is blocked and it is stabilized in the cytoplasm and enters the nucleus to regulate the transcription of Wnt target genes.

There are at least two  $\beta$ -catenin-independent pathways: the planar cell polarity (PCP) pathway and the Ca<sup>2+</sup> pathway (Figure 2). The PCP pathway was originally identified in *Drosophila* where it regulates tissue polarity and cell migration [10, 57]. This signaling pathway requires Fz receptors and Dvl and activates small GTPases including Rho and Rac and the protein kinase JNK. This pathway is also known as the Wnt/JNK pathway. The activation of the Wnt/Ca<sup>2+</sup> pathway triggers the increase in intracellular Ca<sup>2+</sup> levels and activates the protein kinases CamKII and protein kinase C (PKC) [10, 58]. It has been suggested that Wnt-mediated Ca<sup>2+</sup> release involves heterotrimeric G proteins since it is inhibited by pertussis toxin [59]. As mentioned 10 Fz receptors are known in mammals. Fz receptors are seven-transmembrane-spanning receptors that belong to the G protein-coupled receptor (GPCR) list as a separate class [60]. Fz receptors have an extracellular amino-terminal region that contains a cysteine-rich domain (CRD) consisting of 120 to 125 residues with 10 conserved cysteines that is relevant for the binding of Wnt proteins [61]. Growing evidence indicate the involvement of G protein in the Wnt/Fz signaling. The first evidence came from inhibition of non-canonical Wnt effects by pertussis toxin [62]. Later on, many reports have indicated that heterotrimeric G protein participates of canonical and non-canonical Wnt signaling in *Drosophila*, *Xenopus* and mammals [63-69].



**Figure 2.  $\beta$ -catenin-independent Wnt signaling pathways.** In the Wnt/JNK pathway or PCP pathway, a Wnt ligand through a Fz receptors and Dvl activates small GTPases including Rho and Rac and JNK, which in turns modulate cytoskeletal organization. The activation of the Wnt/ $\text{Ca}^{2+}$  pathway triggers an increase in intracellular  $\text{Ca}^{2+}$  levels which activates CamKII and PKC.

### 3. Roles of the Wnt signaling pathway at central synapses

The Wnt signaling pathway has different roles during development linked to neurite patterning and synaptogenesis. Different Wnt ligands have been linked to the presynaptic assembly. In 1997, Salinas and co-workers demonstrated in cerebellar neurons that Wnt-7a increases the levels of synapsin I, a protein associated to synaptic vesicles [70]. Moreover, Wnt-7a mutant mice show a delay in the accumulation of synapsin I [71]. In hippocampal neurons Wnt-7a as well as Wnt-3a and Wnt-7b increases the number of pre-synaptic puncta suggesting a role for these ligands in presynaptic assembly [18, 72, 73]. In addition, Wnt-7a was found to stimulate recycling and endocytosis of synaptic vesicles using FM dyes [74]. In hippocampal neurons, Wnt-7a was also able to increase the expression as well as the clustering of the  $\alpha 7$ - nicotinic acetylcholine receptor ( $\alpha 7$ -nAChR), indicating that the Wnt signaling regulates the clustering of presynaptic receptors [75]. Interestingly, all these ligands are able to modulate presynaptic differentiation by activation of the Wnt/ $\beta$ -catenin signaling pathway, suggesting that some of the components associated with this pathway may be involved in the presynaptic effect. On the other hand, the non-canonical ligand Wnt-5a decreases the number of presynaptic



terminals [72], indicating that canonical and non-canonical signaling pathways may have promoting and inhibitory effects on presynaptic differentiation respectively. In accordance, electrophysiological recordings on adult rat hippocampal slices showed that Wnt-7a, but not Wnt-5a, increased neurotransmitter release in CA3-CA1 synapses by decreasing paired pulse facilitation and increasing the frequency of miniature excitatory postsynaptic currents (mEPSC) [73]. Also, Wnt-7a/Dvl1 double mutant mice exhibit decreased mEPSC frequency at the mossy fiber-granule cell synapse revealing a defect in neurotransmitter release [18].

The Wnt signaling also plays relevant roles in the postsynaptic structure. Wnt-5a, which activates non-canonical Wnt signaling cascades in hippocampal neurons [19, 76], modulates postsynaptic assembly by increasing the clustering of the postsynaptic density protein-95 (PSD-95) and increases spine morphogenesis in cultured hippocampal neurons [15, 19]. PSD-95 is a scaffold protein of the postsynaptic density (PSD), which is a multiprotein complex that interacts with key molecules involved in the regulation of glutamate receptor targeting and trafficking and regulatory proteins relevant for neurotransmission [77, 78]. In hippocampal neurons, Wnt-5a induces a fast increase in the number of clusters of PSD-95 without affecting total levels of PSD-95 protein or presynaptic protein clustering [19]. This postsynaptic effect is dependent on Wnt/JNK signaling pathway as demonstrated by using JNK inhibitors. In long-term experiments, we observed that Wnt-5a is also able to increase the total number of synapses [79]. When hippocampal neurons were incubated with the formylated hexapeptide Foxy-5, which is derived from the sequence of Wnt-5a and mimics the full Wnt-5a molecule action in neurons and other systems [19, 80], there was an increase in PSD-95 since 1 hour, but after 24 hours an increase in the synaptic vesicle protein 2 (SV2) clustering was also observed. In consequence, there was an increase in the total number of synaptic contacts [79].

Also, we determined that Wnt-5a induced a transient formation of dendrite protrusions that resulted in a net increase of mature dendrite spines. Videomicroscopy revealed that Wnt-5a induced *de novo* formation of dendritic spines and also increased the size of the preexisting ones [15]. Interestingly, treatment with the soluble CRD region of Fz2, acting as a Wnt scavenger, decreased spine density in cultured neurons, supporting the physiological relevance of this finding and supporting the implication of Wnt ligands in dendrite spine morphogenesis. Wnt-7a is also able to increase the density and maturity of dendritic spines through a CamKII-dependent mechanism [81]. Wnt-7a rapidly activates CaMKII in spines and inhibition of this kinase abolishes the effects of Wnt-7a on spine growth and excitatory synaptic strength. This finding implicates the Wnt/Ca<sup>2+</sup> signaling cascade in synaptic effects of Wnt ligands. Interestingly, Wnt-5a and Wnt-7a induces an increase in intracellular Ca<sup>2+</sup> concentration [15, 81], supporting the activation of this non-canonical Wnt pathway.

In addition to the structural effects of Wnt ligands at the excitatory synapse, different Wnts have shown modulatory effects on glutamatergic neurotransmission. Wnt-3a modulates the recycling of synaptic vesicles in hippocampal synapses [73, 82] and is able to induce an increase in the frequency of mEPSC [20]. In hippocampal slices, blockade of Wnt signaling impairs long-term potentiation (LTP), whereas activation of Wnt signaling facilitates LTP [17]. In the case of Wnt-5a, acute application of this ligand in hippocampal slices increases the amplitude of field excitatory postsynaptic potentials (fEPSP) and upregulates synaptic NMDA receptor

currents facilitating induction of LTP [15, 16]. Interestingly, Wnt-5a produced a two-step increase in the amplitude of NMDAR responses [16]. The mechanisms involved in this two-step effect of Wnt-5a were investigated by the delivery of specific protein kinase inhibitors via the recording pipette. Specifically, the role of PKC and JNK was investigated, since these are two known downstream kinases of the non-canonical pathway. Inhibition of Ca<sup>2+</sup>-dependent PKC isoforms with Go6976 or the more general PKC inhibitor calphostin C eliminated the first step of potentiation of NMDAR currents and did not affect the second one. On the contrary, the slower developing increase in NMDAR currents was blocked by the JNK inhibitors TJIP153-163 and SP600125. This indicates that there are two mechanisms involved in the potentiation of NMDAR by Wnt-5a. There is a fast PKC-dependent potentiation and a slower JNK-dependent potentiation that does not require previous activation of PKC [16].

Wnt-5a also regulates postsynaptically the hippocampal inhibitory synapses [76]. *Wnt-5a* induces surface expression and maintenance of GABA<sub>A</sub> receptor in the membrane of hippocampal neurons, increases the amplitude of GABA-currents due to a postsynaptic mechanism, and induces the recycling of functional GABA<sub>A</sub> receptors through activation of CaMKII [76]. Therefore Wnt-5a is able to modulate both, excitatory and inhibitory synapses which must be relevant for neurotransmission.

The novel role for Wnt ligands in synaptic transmission provides a mechanism for Wnt signaling to acutely modulate synaptic plasticity and brain function in later stages of development and in the mature organism. Importantly, neuronal activity modulates the release and expression of Wnt ligands which may be relevant for the function of these ligands during neurotransmission. Activation of NMDA receptors increases the expression of Wnt-2 in hippocampal neurons which then stimulates dendritic arborization [83]. On the other hand, tetanic stimulation induces NMDA receptor-dependent synaptic Wnt3a release [17]. The role for endogenous Wnts was supported by incubation of hippocampal slices with secreted Wnt inhibitors, such as secreted Frizzled-related protein-2 (sFRP-2), which showed that endogenous Wnt ligands are modulators of glutamatergic neurotransmission being necessary to maintain basal NMDA receptor synaptic transmission [15, 16].

The *in vivo* relevance for the role of Wnt signaling in activity-mediated synaptic connectivity was revealed in mice exposed to an enriched environment (EE). These animals showed increased complexity and number of large mossy fiber terminals in the CA3 region [84]. EE increased Wnt7a/b levels in CA3 pyramidal neurons and inhibiting Wnt signaling through locally applied sFRP-1, suppressed the effects of EE on synapse numbers and further reduced synapse numbers in control mice.

These findings show that Wnt ligands are important regulators of the synaptic structure during development and in adult neurons, and that the Wnt pathway is one of the signaling cascades regulated by neuronal activity that is involved in the regulation of neurotransmission in adult nervous system.

In addition to the role of Wnts, Fz receptors have also been involved in synaptic structure and function. In the hippocampus, we have determined that different Fz receptors have very different patterns of expression during development, being some of them highly expressed in

adulthood and others during early development [85]. In addition, the distribution of Fzs in hippocampal neurons is also very specific. Some receptors, are located in the synaptic region, while others are mainly located in the soma or in the growth cones of young neurons [85]. These findings suggest that these receptors could be important regulators for the specific activation of the Wnt signaling cascades during the development of hippocampal circuits. In fact, we determined an association of the distribution with specific functions. In hippocampal neurons, Fz1 is located in the synaptic region co-localizing with presynaptic proteins and with active synaptic vesicle recycling sites [82]. Interestingly, overexpression of Fz1 increased the number of clusters of Bassoon, a component of the active zone involved in the structural organization of neurotransmitter release sites that is recruited early during synapse formation [86], suggesting that Fz1 regulates synaptic differentiation. In agreement, treatment with the extracellular CRD of Fz1 decreased Bassoon clustering which was not observed with the CRD of Fz2, indicating a receptor specificity for the synaptic effect [82]. Fz5 also has a role in mature neurons where it modulates the synaptogenic effect of Wnt7a [87]. As well as Fz1, Fz5 is present in synaptosomes and colocalizes with synaptic markers, and changes in the expression of this receptor modulates the density of synaptic sites [87]. In addition to its function in mature neurons, Fz5 was shown to be in high levels in the growth cones of developing hippocampal neurons [85], and we have recently determined that this receptor is involved in neural polarization (unpublished results). We determined that overexpression of Fz5 triggers a mislocalization of axonal proteins such as Tau-1 and phosphorylated MAP1B (MAP1BP), which change their distribution to the whole cell suggesting altered polarization. When the expression of Fz5 is knocked-down by shRNA, MAP1BP is not polarized and is almost completely lost. These findings suggest that in developing hippocampal neurons Fz5 is relevant for neural polarization. These studies indicate that Fz receptors are relevant players in both the developing and the adult nervous system and support the notion that the Wnt signaling pathway is crucial for different aspects of the development and function of the CNS.

#### **4. Role of Wnt signaling in adult neurogenesis**

In the adult brain, there are two regions where there is a continuous generation of new neurons (Figure 3A), the subventricular zone (SVZ) of the lateral ventricles [88] and the subgranular zone (SGZ) in the hippocampal dentate gyrus [89]. In the SVZ, astrocyte-like neural stem cells (NSCs), called type B1 cells, generate type C cells that rapidly proliferate and give rise to type A neuroblasts (Figure 3B). These cells migrate through the rostral migratory stream to the olfactory bulb where they became interneurons [88] (Figure 3A). In the SGZ, radial and non-radial neural precursor cells give rise to transient amplifying progenitors that generate neuroblasts and then became immature neurons that extend dendrites toward the molecular layer and project their axons through the hilus toward the CA3 region [90] (Figure 3C). Newborn neurons then mature and fully integrate into the preexisting hippocampal circuitry.

Adult neurogenesis is highly regulated by intrinsic and extrinsic mechanisms. Many signaling pathways have been identified as regulators of different aspects of neurogenesis. Notch, Shh,

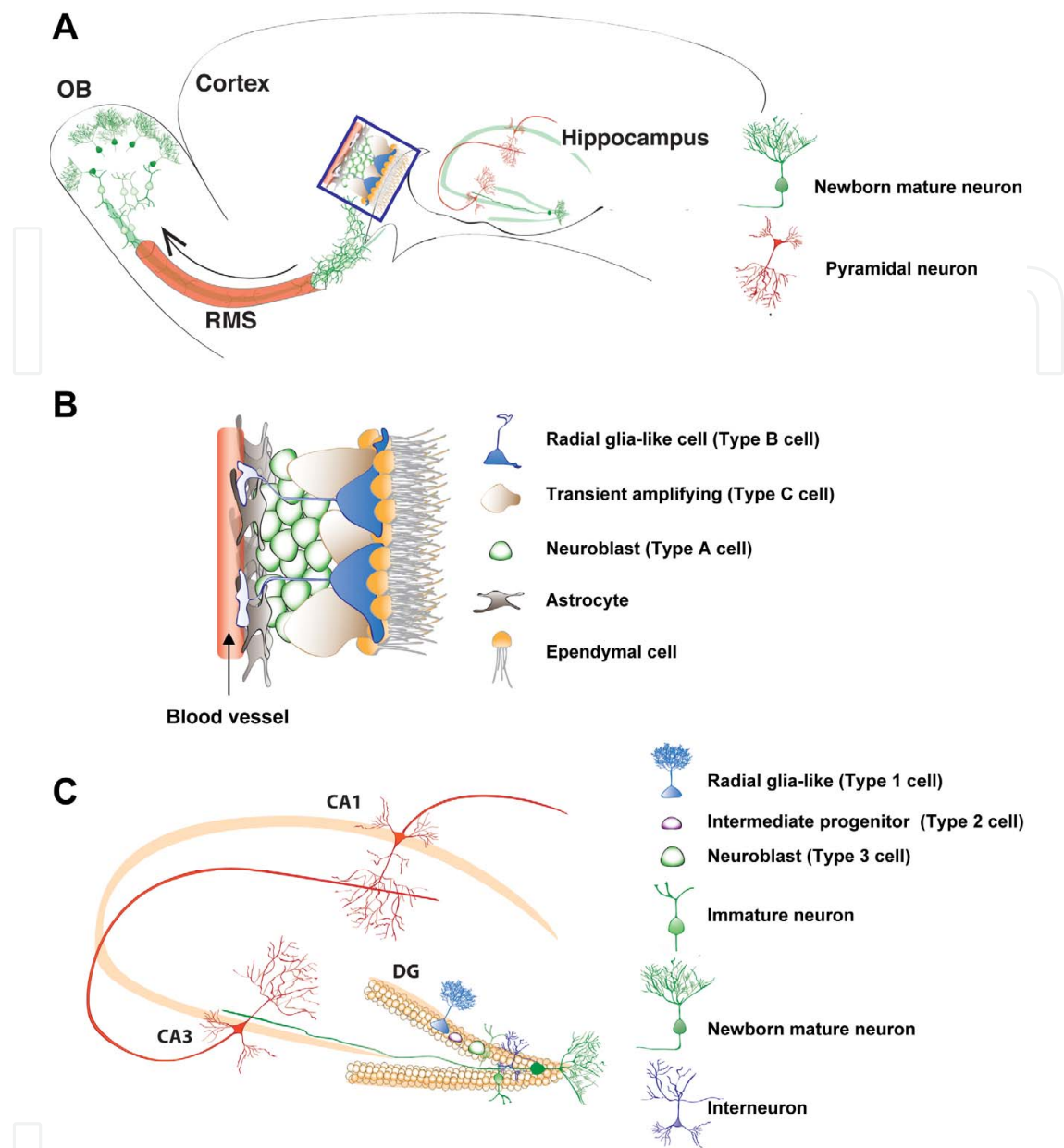


BMPs, and Wnts are part of the signaling molecules of the niche that regulate the maintenance, activation and fate specification of neural precursor cells [91, 92].

In Wnt/ $\beta$ -catenin reporter mice (BATGAL) it was shown that this pathway is active in the SGZ and the dentate granule cell layer [23]. In that study, authors determined that Wnt3 is expressed in adult hippocampal astrocytes and that adult hippocampal progenitor (AHP) cells express key components of the Wnt/ $\beta$ -catenin signaling pathway. These findings suggested that the Wnt pathway may be involved in the regulation of adult neurogenesis. *In vitro* analysis in cultured cells revealed that Wnts derived from hippocampal astrocytes stimulate Wnt/ $\beta$ -catenin signaling in isolated AHPs inducing their neuronal commitment [23]. The effect of the Wnt signaling was supported *in vivo* using lentiviral vectors expressing Wnt3a or a secreted mutant Wnt1 protein that blocks Wnt signaling. Lentiviruses were stereotactically injected into the dentate gyrus of rats. As assessed by the incorporation of the nucleotide analog BrdU and immunodetection of the immature neuron protein doublecortin (DCX), blocking the Wnt signaling decreases adult hippocampal neurogenesis while stimulating this pathway has the opposite effect [23]. More recently, and by using the same lentiviral approach to block Wnt signaling in the dentate gyrus of adult rats it was shown that Wnt-mediated adult hippocampal neurogenesis contributes to learning and memory [93]. In the SVZ,  $\beta$ -catenin signaling also plays a role in the proliferation of progenitor cells in the adult mouse brain [94]. Retrovirus-mediated expression of a stabilized  $\beta$ -catenin promoted the proliferation of type C cells and inhibited their differentiation into neuroblasts. Also in the SVZ, transduction of the  $\beta$ -catenin inhibitor axin by intracranial lentiviral delivery decreased cell proliferation as revealed by decreased BrdU labeling [95], further supporting a role for Wnt/ $\beta$ -catenin signaling in neural stem cell proliferation in the neurogenic areas of adult brain.

The Wnt-mediated effects in neurogenesis may be caused by the transcriptional activation of NeuroD1 which is dependent on the Wnt/ $\beta$ -catenin signaling activation [25]. NeuroD1 is a basic helix-loop-helix transcription factor important for the generation of granule cell and olfactory neuron in the embryonic and adult brain [96]. NeuroD1 gene promoter has overlapping DNA-binding site for Sox2 and TCF/LEF, then the activation of this gene implies activation of the canonical Wnt pathway and removal of Sox2 repression from the NeuroD1 gene promoter [25]. More recently, Prox1 was also determined as a target of the Wnt/ $\beta$ -catenin pathway relevant for neurogenesis [22]. Prox1 is expressed in newborn and mature granule cells and is required for the proper differentiation and survival of newborn granule cells, but not for the maintenance of granule cells after they have fully matured [22].

In addition, Wnts could indirectly modulate adult neurogenesis thorough their effects on neuronal activity. As previously described, different Wnts regulate glutamatergic neurotransmission, and evidence indicates that neural progenitor cells respond to neuronal activity as part of their differentiation program [97]. GABA is an important modulator of adult hippocampal neurogenesis being critical for the proper development and maturation of adult-born neurons [98-100]. Interestingly, Wnt-5a through activation of CaMKII, induces the recycling of functional GABA<sub>A</sub> receptors on hippocampal neurons and modulates inhibitory synapses [76].



**Figure 3. Neurogenesis in the adult brain.** (A) Schematic representation of adult rodent brain highlighting the two neurogenic regions. The hippocampus and the SVZ (boxed). (B) Schematic of the SVZ in the wall of the lateral ventricles. Distinct stem/progenitor cell types (types B, C, and A) are shown. (C) Neurogenesis in the SGZ of the hippocampal dentate gyrus. The progression of radial type 1 cells to mature newborn granule neurons is schematized.

As mentioned, in neurogenic niches Wnts are provided by astrocytes [23], and during aging it was reported that the levels of Wnt3 protein and the number of Wnt3-secreting astrocytes declines [101], which may be one of the factors underlying the impairment of neurogenesis that is observed in aging [102, 103]. On the contrary, running, that is a potent stimulator of adult neurogenesis in the SGZ [104] was found to significantly increase *de novo* expression of Wnt-3 [101], pointing to the Wnt pathway as one of the factors involved in running-mediated increase in neurogenesis. In addition to astrocytes-derived Wnts, an autocrine Wnt signaling activity has been observed in adult hippocampal progenitors (AHPs) derived from adult rat

brains. Inhibiting this autocrine Wnt signaling increases the number of neurons formed and leads to a loss of multipotency among AHPs indicating that this autocrine pathway may preserve the balance between neural stem cell maintenance and differentiation [105].

The Wnt signaling has also been involved in the mechanism of the orphan nuclear receptor TLX (also known as NR2E1), which is an important regulator of neural stem cell maintenance and self-renewal in embryonic and adult brains [106, 107] and is involved in neurogenesis in the SVZ [108] and hippocampus [109]. To stimulate neural stem cell proliferation and self-renewal TLX activates the Wnt/ $\beta$ -catenin pathway in adult mouse neural stem cells by activating the expression of Wnt-7a, which expression was found to be downregulated in TLX-null mice, through binding to two TLX binding sites present in the Wnt-7a gene promoter [95]. Wnt-7a is important for adult neural stem cell proliferation *in vivo* since there is a decreased BrdU labeling in the SGZ and SVZ of adult Wnt7a knockout mice. In TLX<sup>-/-</sup> mice, intracranial lentiviral transduction of active  $\beta$ -catenin led to a considerable rescue of cell proliferation in the SVZ, suggesting that Wnt/ $\beta$ -catenin acts downstream of TLX to regulate neural stem cell proliferation *in vivo* [95].

It has been shown that low oxygen is associated with increased levels of  $\beta$ -catenin *in vivo*, and that hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) modulates the Wnt/ $\beta$ -catenin signaling in embryonic stem cells exposed to low oxygen [110]. Recently, we determined *in vivo* that hypoxia stimulates the activation of the Wnt/ $\beta$ -catenin signaling pathway in the hippocampus of adult mice (our unpublished results), and stimulates cell proliferation in the SGZ of 2 month old wild-type mice.

Altogether, these findings indicate that the Wnt pathway is relevant not only for the development of the nervous system but also for the development of new neurons in the adult brain, being important for the maintenance and self-renewal of the stem cell pool and for the commitment and proliferation of new neurons.

## 5. Wnt signaling in Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive deterioration of cognitive abilities, cerebral accumulation of extracellular amyloid plaques composed mainly of amyloid- $\beta$  peptide (A $\beta$ ), and synaptic alterations [111]. In addition to the accumulation of A $\beta$  aggregates, which is a product of the processing of the amyloid precursor protein (APP), cytoskeletal alterations associated to the abnormal phosphorylation of the microtubule associated protein tau [112, 113] are early manifestations that lead to aberrant remodeling of dendrites and axons, the appearance of dystrophic neurites, synaptic loss [114], and eventually progressive loss of neuronal populations [112].

During more than a decade, a strong relationship between an impaired Wnt signaling pathway activity and neuronal damage in AD has been raised [31, 115-118]. Different studies have shown that Wnt signaling components are altered in AD [119-124], and in addition, the Wnt signaling pathway has been related to other neurodegenerative disorders such as autism and

schizophrenia [30, 125]. Among the Wnt components that are affected in AD, it was shown that  $\beta$ -catenin levels are reduced in AD patients carrying presenilin-1 (PS-1)-inherited mutations [124], while the secreted Wnt antagonist Dickkopf-1 (Dkk1) is elevated in postmortem AD brains and brains from transgenic mouse models for AD [121, 126]. A variant of the LRP6 has been associated with late-onset AD, which confers low levels of Wnt signaling [119]. In addition, genetic studies show a link between Wnt signaling and AD. Epidemiological data show an increased risk for AD in populations where the allele 4 of apo-lipoprotein E (apoE4) is present. Interestingly apoE4 causes inhibition of the canonical Wnt signaling in PC12 cells upon stimulation with Wnt-7a as determined by luciferase activities and nuclear  $\beta$ -catenin levels [127]. A $\beta$  directly binds to the extracellular CRD of Fz5 at or in close proximity to the Wnt-binding site inhibiting the canonical Wnt signaling pathway [128], linking directly A $\beta$  to Wnt impairment. Moreover, the exposure of cultured rat hippocampal neurons to A $\beta$  results in inhibition of canonical Wnt signaling as determined by destabilization of endogenous levels of  $\beta$ -catenin, increase in GSK-3 $\beta$  activity, and a decrease in the expression of some Wnt target genes [129]. Moreover, acute exposure to A $\beta$  increases Dkk1 mRNA levels in hippocampal brain slices, which seems to be associated to synaptic loss induced by A $\beta$  [130].

As mentioned, one of the hallmarks of AD brains is the abnormal phosphorylation of the tau protein which accumulates as intraneuronal neurofibrillary tangles [131]. Several kinases can phosphorylate tau *in vitro*; however, the bulk of the information supports that Cdk5, extracellular signal-related kinase 2, microtubule affinity-regulating kinase and GSK-3 $\beta$ , a key component of the Wnt cascade, are the most relevant kinases for tau phosphorylation *in vivo* [132, 133]. Cultured neurons exposed to A $\beta$  show an increased GSK-3 $\beta$  activity [134, 135], and active GSK-3 $\beta$  has been found in brains staged for AD neurofibrillary changes, with a concomitant decrease in  $\beta$ -catenin levels and an increase in tau hyperphosphorylation [136]. Also, neurodegeneration and spatial learning deficits have been observed in GSK-3 $\beta$  conditional transgenic mice [137, 138]. Interestingly, a study shows that the phosphorylation of tau antagonizes apoptosis by stabilizing  $\beta$ -catenin; therefore, up-regulation of  $\beta$ -catenin during tau phosphorylation prevents the cell from going into apoptosis. Increasing levels of phosphorylated tau was correlated with increased levels of nuclear  $\beta$ -catenin, and the knockdown of  $\beta$ -catenin antagonizes the anti-apoptotic effects of tau [139]. These findings support a role of  $\beta$ -catenin as a survival element in AD.

Several studies have shown neuroprotective properties of the Wnt signaling activation against the toxicity of A $\beta$  peptide. In cultured hippocampal neurons, exposure to A $\beta$  aggregates causes a decrease in endogenous  $\beta$ -catenin levels, and this effect was overcome by direct activation of the pathway with Wnt-3a conditioned media [117, 129]. The protective effect of Wnt-3a against the toxicity of A $\beta$  oligomers was shown to be mediated by Fz1 receptor, since this effect is modulated by the expression levels of Fz1 in both, PC12 cells and hippocampal neurons [14]. Overexpression of Fz1 significantly increased cell survival induced by Wnt-3a and diminished caspase-3 activation, while knocking-down the expression of the receptor by antisense oligonucleotides decreased the stabilization of  $\beta$ -catenin induced by Wnt-3a and decreased the neuroprotective effect elicited by this Wnt ligand [14].



In agreement with the effect of Wnt-3a, inhibition of GSK-3 $\beta$  by lithium protects hippocampal neurons from A $\beta$ -induced damage. More importantly, *in vivo* lithium treatment of double transgenic APP<sup>swe</sup>/PSEN1 $\Delta$ E9 mice, which is a well characterized *in vivo* model of AD that shows most hallmarks of the disease [140], reduced spatial memory impairment, decreased A $\beta$  oligomers and the activation of astrocytes and microglia [141]. *In vivo*, lithium treatment activated the Wnt signaling as shown by the increase in  $\beta$ -catenin and by the inhibition of GSK-3 $\beta$  [141]. These studies suggest that the loss of normal Wnt/ $\beta$ -catenin signaling activity may be involved in the A $\beta$ -dependent neurodegeneration observed in AD and that the activation of the pathway might have beneficial effects for the treatment of the disease [12].

APP<sup>swe</sup>/PSEN1 $\Delta$ E9 mice show decreased levels of adult neurogenesis [142]. In these mice, we evaluated the effect of hypoxia on the generation of new neurons in the hippocampus. As previously mentioned hypoxia induces the activation of the Wnt/ $\beta$ -catenin signaling pathway in the hippocampus of wild-type mice. Mice were exposed to low oxygen and neurogenesis was evaluated by incorporation of BrdU and double staining with DCX. It was determined that hypoxia is a strong stimulator of neurogenesis in AD mice (our unpublished results). Currently we are evaluating whether this effect is related to the activation of the canonical Wnt pathway. Also, we have observed that voluntary wheel running strongly increased neurogenesis in APP<sup>swe</sup>/PSEN1 $\Delta$ E9 mice and also decreased A $\beta$  burden and tau phosphorylation (our unpublished results). As previously mentioned, voluntary running was found to increase *de novo* expression of Wnt-3 [101], suggesting that the effects observed in runner AD mice could involve the activation of the Wnt signaling pathway.

In addition to the role of the canonical Wnt signaling, we have studied whether Wnt-5a is able to protect neurons against A $\beta$  oligomers synaptotoxicity [143]. Synaptic failure is an early event in AD, and soluble A $\beta$  oligomers are proposed to be responsible for the synaptic pathology that occurs before the plaque deposition and neuronal death [74, 144]. Electrophysiological analysis of Schaffer collaterals-CA1 glutamatergic transmission in hippocampal slices demonstrated that Wnt-5a prevents the decrease in the amplitude of fEPSP and EPSCs induced by A $\beta$  oligomers, indicating that Wnt-5a prevents the synaptic damage triggered by A $\beta$  [143]. Moreover, Wnt-5a prevented the decrease in the postsynaptic density scaffold protein PSD-95 and synaptic loss in cultured hippocampal neurons [143], supporting that Wnt-5a improves synaptic function in the presence of A $\beta$ .

Additionally, the activation of several signaling pathways that crosstalk with the Wnt pathway also supports the neuroprotective potential of the Wnt cascades in AD [12].

## 6. Conclusions

As we have discussed throughout this Chapter, the Wnt signaling pathway has fundamental roles in the development and function of the CNS. As discussed, the canonical and non-canonical Wnt signaling cascades have shown to be important for the formation and structure of central synapses, and in addition to the structural effects, Wnt ligands acutely modulate synaptic transmission and plasticity. Also, in the adult brain the Wnt pathway is one of the



signaling cascades that regulates the generation of new neurons in neurogenic niches. Importantly, different stimuli that regulate neurogenesis involve the regulation of the Wnt signaling, implicating this pathway as a relevant player in the modulation of this physiological process.

Considering all the discussed roles of Wnts, it was expected that alterations in the Wnt cascades leads to diseases associated to the nervous system. In fact, deregulation of the Wnt pathway has been related to mental disorders, mood disorders and neurodegenerative diseases. As we have discussed, a bulk of evidence associate Wnt dysfunction to AD, and strongly point to a neuroprotective potential of the Wnt cascades as a therapeutic approach. Future work should focus on explore the therapeutic benefits of stimulating the Wnt signaling pathway *in vivo*.

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## Author details

Nibaldo C. Inestrosa and Lorena Varela-Nallar

Center for Aging and Regeneration (CARE), Department of Cell and Molecular Biology, Faculty of Biological Sciences, Pontifical Catholic University of Chile, Santiago, Chile

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